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Rato, Márcia; Bexiga, Ricardo; Florindo, Carlos; Nunes, Sandro F.; Cavaco, Lina; Vilela, Cristina L.; Santos-Sanches, Ilda

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MOLECULAR EPIDEMIOLOGY OF MACROLIDE AND/OR TETRACYCLINE RESISTANT *STREPTOCOCCUS AGALACTIAE* AND *STREPTOCOCCUS UBERIS* FROM BOVINE MASTITIS

Márcia Rato¹, Ricardo Bexiga², Carlos Florindo³, Sandro F. Nunes^{2,4}, Lina M. Cavaco^{2,5}, Cristina L. Vilela², Ilda Santos-Sanches¹

¹CREM, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; ²CIISA, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Lisboa, Portugal. ³Departamento de Doenças Infecciosas, Instituto Nacional de Saúde, Lisbon, Portugal; ⁴Presently at Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK, ⁵Presently at National Food Institute, Faculty of Life Sciences/ Technical University of Denmark, Copenhagen, Denmark

Objectives: To identify macrolide/tetracycline phenotypes and genotypes among *Streptococcus agalactiae* (Group B Streptococcus, GBS) and *S. uberis* from bovine subclinical mastitis, relevant for therapeutic policies, and evaluate further molecular features.

Methods: GBS (n=60) and *S. uberis* (n=30) field isolates from 9 herds in Portugal were characterized by pulsed field gel electrophoresis (PFGE). Resistance to macrolides (erythromycin-E), lincosamides (pirimycin-PRL), tetracycline-TET, and the constitutive macrolide-lincosamide resistance phenotype (cMLS) was evaluated by disk diffusion. Resistance genes (*mefA*; *ermA*; *ermB*; *linB*; *tetM*; *tetO*; *tetT*; *tetS*; *tetQ*; *tetK*; *tetW*; *tetL*) were screened by PCR among all isolates. The C5a peptidase and *lmb* genes, important virulence factors in human GBS localized in a composite transposon, were screened by PCR among the bovine GBS. Capsular serotyping of GBS was performed by agglutination and by PCR-sequencing the capsule gene cluster.

Results: A total of four PFGE clusters comprised 50% of the GBS and four PFGE clusters comprised 53% of the *S. uberis*. Co-resistance to E and PRL (18%-27%) and to TET (57%-60%) was observed in both species. Resistance to PRL and susceptibility to E (LSA phenotype) was found in 27% of *S. uberis* isolates. Diverse resistance genotypes were found: *ermB/tetO/tetK* in GBS and *ermB/tetO* or *linB/tetS* in *S. uberis*. Both C5a peptidase and *lmb* genes were present in 20% of GBS. Two known molecular serotypes were detected: III-3 (n=1) and V (n=14), and four types (detected among 75% of GBS) showed *cpsDEF* sequences distinct from those described so far, and most were found to be herd-specific.

Conclusion: A contagious source for both GBS and *S. uberis* was assessed. Dissemination of antimicrobial-resistance was clonal and by lateral gene transfer. Serotype-specific capsular polysaccharides appear to have evolved in a herd-associated manner. GBS with C5a peptidase and *lmb* were of capsular serotype V –common in human GBS infection.

297 words

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